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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)* Breast cancer osteolysis is common and the morbidity is devastating. The consequences of intractable bone pain, fracture, hypercalcemia and nerve compression syndromes are debilitating and the tumor is incurable once it has metastasized to bone. Women with bone metastases live many years with this incurable complication and are at high risk for morbidity. A more aggressive approach to prevent and treat bone metastases is a necessary addition to the standard armamentarium for breast cancer therapy in order to impact on this morbidity. Although bisphosphonates are now FDA-approved for treatment of established bone metastases and have had significant impact on bone pain and fracture[6], considerable advances are necessary for the eventual prevention or reversal of bone metastases. These data indicate a role for TGFβ to potentiate ER-α-mediated transcription induced by a constitutively active ER-α. The above in vitro studies provide rationale for targeting the downstream effects of TGFβ on breast cancer cells to treat and eventually prevent osteolysis. However, in vivo, expression of wild-type ER-α and mutants Ser47Thr, Lys531Glu, and Tyr537Asn had no effect on bone metastases in a mouse model. Thus, further experiments to test the in vivo relevance of these in vitro findings are warranted.

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INTRODUCTION

Breast cancer commonly metastasizes to the skeleton in patients with advanced disease to cause either bone destruction (osteolytic metastases) or new bone formation (osteoblastic metastases) and significant morbidity[3,5]. Since patients with breast cancer may survive several years with their bone metastases, it is important to understand the pathophysiology of this process in order to improve therapy and prevention. The proposed work seeks to investigate tumor cell-bone interactions in breast cancer metastases to bone with specific attention to the role of 1) estrogen receptor- (ER-α) in mediating tumor production of bone-active factors to cause osteolytic and osteoblastic metastases using a mouse model of bone metastases and 2) bone-derived transforming growth factor (TGFβ) in modulating the effects of ER-α on tumor cell growth in bone. A constitutively active ER-α (Tyr537Asn), identified from a bone metastases[10], when expressed in human breast cancer cells is associated with increased production of parathyroid hormone-related protein (PTHrP), a stimulator of osteolytic metastases. Furthermore, TGFβ enhances the ER-α mediated transcriptional activity induced by the Tyr537Asn in human breast cancer cells. Defining the mechanisms responsible for breast cancer metastases to bone will provide insight into future therapy and prevention. ***The following hypotheses will be tested:***

1. Estrogen stimulates breast cancer cell production of factors which disrupt normal bone remodeling to result in osteolytic or osteoblastic metastases.

2. Estrogen stimulates PTHrP production by TGFβ-responsive breast cancer cells to result in osteolytic metastases. TGFβ enhances ER-α mediated transcriptional activity in breast cancer cells to stimulate growth.

3. Estrogen stimulates production of osteoblastic factors, such as ET-1, by breast cancer cells which are TGFβ unresponsive. Restoration of TGFβ responsiveness should result in PTHrP production and osteolytic metastases.

The following specific aims are proposed to test the hypotheses:

1. To determine the role of ER-α in osteolytic or osteoblastic breast cancer metastases to bone using an in vivo model. Wild-type ER-α and various mutants (Ser47Thr, Lys531Glu, and Tyr537Asn) will be stably transfected into breast cancer cell lines which are known to cause either osteolytic or osteoblastic metastases in a mouse model (MDA-MB-231, ZR-75-1, MCF-7, T47D, MDA-MB-468) as well as into cell lines which are tumorigenic in nude mice but do not cause bone metastases and clonal lines isolated. In vitro growth, PTHrP production, ET-1 production, TGFβ responsiveness, ER-α mediated transcriptional activity and effect of exogeneous estrogens and antiestrogens will be tested in stable cell lines. In vivo, the effect of expression of wt ER-α or mutants on bone metastases will be studied in a mouse model.

2. To determine if the effect of TGFβ to increase ER-α mediated transcriptional activity is specific for the constitutively active ER-α Tyr537Asn mutant compared with wt ER-α or is cell-specific.

Stable MDA-MB-231 cell lines expressing ER-α mutants or wt will be treated with TGFβ, with or without estrogens or antiestrogens. ER-α transcriptional activity will be assessed by transient transfection with ERE-luc and PTHrP secretion into conditioned media will be assessed by immunoradiometric assay. ER-α mutants (Ser47Thr, Lys531Glu, and Tyr537Asn) or wt will be stably expressed in ER-α positive cell lines (ZR-75-1, MCF-7 (both lines), and T47D) and assessed as in the MDA-MB-231 stable constructs.

3. To determine the relationship between TGFβ signaling and ER-α mediated transcription.

Specific molecular aspects to be addressed include 1) whether these effects are mediated through the known TGFβ serine-threonine kinase-Smad signaling pathway and 2) whether TGFβ enhances production of nuclear receptor coactivators of ER-α response, such as AIB1 or SRC-1 to enhance

ER-α dependent transcription. ER-α mutants (Ser47Thr, Lys531Glu, and Tyr537Asn) or wt will be transiently transfected into stable MDA-MB-231 clones which stably express one the following components of the TGFβ receptor-signaling pathway: truncated (dominant-negative) type II receptor, constitutively active type I receptor, Smad2 dominant-negative along with ERE-luciferase reporter construct. Cells will be treated with TGFβ and ER-α mediated transcriptional activity will be assessed. Western blots will be performed on cell lysates for nuclear coactivators of ER-α response, AIB1 and SRC-1. In each specific aim, ER-α positive (MCF-7) and ER-α negative (MDA-MB-468) cell lines in which both TGFβ responsive and -unresponsive sublines exist will be used to assess the interaction of TGFβ-ER-α within the same cell line.

BODY

The research accomplishments completed during year 1 are described according to the approved statement of work . Tasks 1-3 were originally scheduled for completion by month 18.

STATEMENT OF WORK

1. To determine the role of ER-α in breast cancer cells which cause osteolytic or osteoblastic metastases. (Months 1-18). Rationale: Women with ER-α positive primary tumors are more likely to develop bone metastases[3,7]. Although scant data suggest that estrogen may regulate PTHrP expression in the uterus[9], and in a breast cancer cell line, there is no clear relationship between PTHrP and ER-α in primary breast cancer. The sparse clinical data available on ER-α expression in breast cancer bone metastases indicate that 60-75% are ER-α negative[4] despite the fact that women with ER-α positive primary tumors are more likely to develop bone metastases. Furthermore, bone metastases were frequently ER-α negative in those patients in whom the primary tumors were ER-α positive[4]. Recently, 3 missense mutations were identified in the ER-α gene from metastatic breast cancer: Ser47Thr, Lys531Glu, and Tyr537Asn[10]. The first 2 ER-α mutants had similar activity to wild-type (wt) ER while the Tyr537Asn ER mutant demonstrated a potent, estradiol-independent transcriptional activity as compared to wt ER-α. This constitutive activity of Tyr537Asn was unaffected by estradiol, tamoxifen or the pure antiestrogen ICI 164,384. This Tyr537Asn mutant was derived from a bone metastases which was ER-α negative by ligand binding analysis. The mutation is located in exon 8 of the carboxy-terminal portion of the hormone-binding domain of the ER-α, a potential phosphorylation site[2] implicated in hormone binding, dimerization, and hormone-dependent transcriptional activity. Such a mutation may be responsible for the development and progression of breast cancer metastases to bone, and since it does not bind ligand, may be classified as an ER-α negative tumor. Since bone metastases are infrequently sampled, the prevalence of this ER-α mutation is unknown. However, the exact mutation has also been identified in an endometrial carcinoma[8]. To determine the role of ER-α in the development and progression of osteolytic metastases, we proposed to express these wild-type ER-α or mutants, Ser47Thr, Lys531Glu, and Tyr537Asn, into the ER-α negative breast cancer cell line, MDA-MB-231 which causes osteolytic bone metastases in a mouse model[1].

Task 1: Wild-type ER-α and various mutants (Ser47Thr, Lys531Glu, and Tyr537Asn) will be stably transfected into breast cancer cell lines which are known to cause either osteolytic or osteoblastic metastases in a mouse model (MDA-MB-231, ZR-75-1, MCF-7, T47D, MDA-MB-468) as well as into cell lines which are tumorigenic in nude mice but do not cause bone metastases and clonal lines isolated.

Stable MDA-MB-231 cell lines were constructed which express wild-type ER-α and mutants Ser47Thr, Lys531Glu, and Tyr537Asn. Over 50 clones of each different ER-α transfectants were

screened by measuring luciferase activity in the presence or absence of 17-estradiol after transient transfection with the estrogen response element linked to luciferase (ERE-luc). Among the wild-type ER-α and ER-α mutants Ser47Thr and Lys531Glu transfectants, at least 5 clones responded to 17-estradiol with a significant increase in ERE-luciferase activity. Six clones were identified from the ER-α mutant Tyr537Asn group which had increased ERE-luciferase activity in the absence of 17-estradiol. These six clones did not respond further to 17-estradiol or the antiestrogen tamoxifen. The stable cell lines were tested in a mouse bone metastases assay. The results are described below under task 3.

We have been unsuccessful in constructing stable cell lines of the other human breast cancer lines, ZR-75-1, MCF-7, T47D and MDA-MB-468 which express wild-type ER-α or ER-α mutants Ser47Thr, Lys531Glu, and Tyr537Asn. In fact, we have been unable to stably express any cDNA in ZR-75-1 and T47D, despite using multiple conditions and transfection methods. MCF-7 cells initially expressed the transfected ER-α constructs, however, clones did not remain stable. Finally, we have successfully constructed clones of MDA-MB-468 which express Smad 4 and we are in the process of testing stability of these clones.

Task 2: In vitro growth, PTHrP production, ET-1 production, TGFβ-responsiveness, ER-α mediated transcriptional activity and effect of exogeneous estrogens and antiestrogens will be tested in stable cell lines.

Figure 1 shows that stable MDA-MB-231 clones which express ER-α (Tyr537Asn) mutant demonstrates increased ER-α mediated transcriptional activity in the absence of estradiol, as assessed by transient transfection with the ERE-luciferase compared with empty vector control. Transcriptional activity of the stable clones was not affected by estradiol treatment, but exogenous TGFβ1 increased ERE-luciferase activity in all stable clones (FIGURE 1). Furthermore, basal as well as TGFβ-stimulated PTHrP secretion by the Tyr537Asn ER mutant clones was increased compared with the empty vector controls (FIGURE 2). These data suggest that ER-α mediated transcription is associated with increased tumor production of PTHrP. This, in combination with the effect of TGFβ to enhance ER-α mediated transcription, and potentially growth, may be a mechanism for the propensity of breast cancer to metastasize to the skeleton.

FIGURE 1: TGFβ enhances ER-α mediated transcriptional activity in stable MDA-MB-231 clones which express the constitutively active ER-α [MDA/ER(Tyr537Asn)]. MDA/ER(Tyr537Asn) and empty vector control clones (MDA/pcDNA3) were transiently transfected with ERE-luciferace reporter, switched to phenol red-free media with charcoal-stripped serum at 8 hr and incubated for 24 hrs more and treated with TGFβ, estradiol or both for 24 hrs. Values represent the mean ± SEM of triplicate measurements. Statistical analysis by ANOVA.

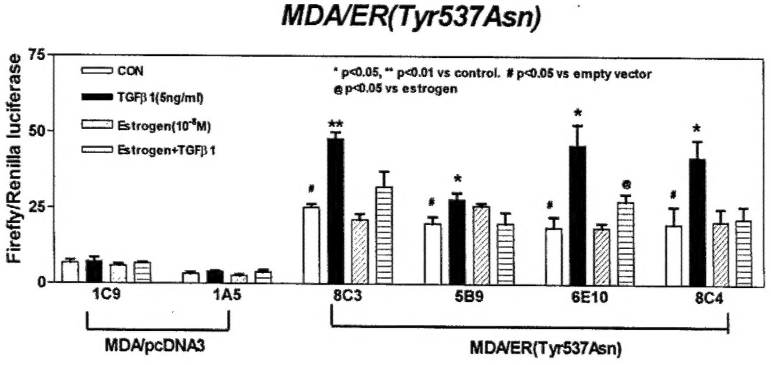
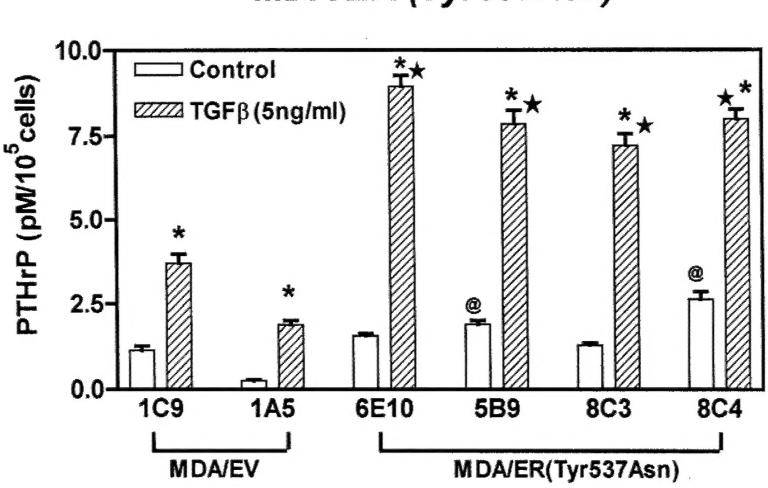


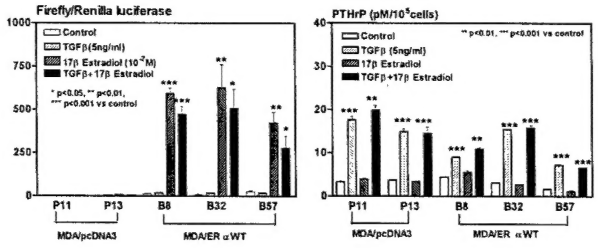
FIGURE 2: TGFβ enhances PTHrP production by MDA-MB-231which express the constitutively active ER-α [MDA/ER(Tyr537Asn)] PTHrP secretion into 24 hour conditioned media obtained from samples illustrated in figure 1. PTHrP was measured by immunoradiometric assay and corrected for cell number. Values represent the mean ± SEM of triplicate measurements. Statistical analysis by ANOVA. MDA/EV=empty vector pcDNA3 clones.



* p<0.01 vs control
@ p<0.05 vs 1C9 control and , p<0.001 vs 1A5 control
★ p<0.01 VS 1C9 and 1A5 TGFβ stimulated PTHrP

To determine if the effects of TGFβ on ER-α mediated transcription were specific to the ER-α (Tyr537Asn) mutant, we constructed stable MDA-MB-231 cell lines which expressed wild-type ER-α, or ER-α mutants which were identified in soft tissue metastases, Ser47Thr and Lys531Glu. These data, (FIGURES 3a-c) illustrate that although ER-α mediated transcription was increased in response to 17-estradiol in clones which expressed wild-type or Ser47Thr and Lys531Glu mutants, there was no additional effect of TGFβ. Furthermore, the combination of 17-estradiol and TGFβ did not increase PTHrP production over TGFβ alone. There was no significant difference between wild-type ER-α and the Ser47Thr or Lys531Glu mutants. These data suggest that the ER-α (Tyr537Asn) mutant, isolated from a bone metastasis, may confer specific properties to the breast cancer cells which facilitate osteolytic bone metastases.

MDA-MB-231
ER-α wild-type



MDA-MB-231
ER-α Lys531Glu

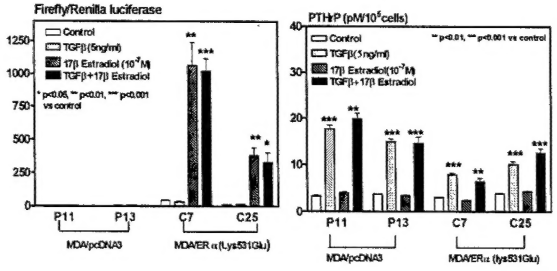
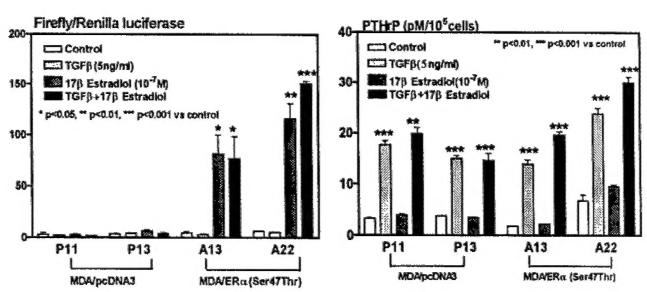


FIGURE 3: Estradiol, but not TGFβ, increase ER-α mediated transcriptional activity (left panel) and PTHrP production (right panel) in stable MDA-MB-231 clones expressing the wild-type ER-α (a) or mutants Ser47Thr (b) and Lys531Glu (c) compared with empty vector control clones (MDA/pcDNA3). Clones were treated with TGFβ, estradiol or both. ERE luciferase activity and PTHrP measurements were assessed as in figure 7 & 8 Values represent the mean ± SEM of triplicate measurements. Statistical analysis by ANOVA.

MDA-MB-231
ER-α Ser47Thr



Task 3: In vivo, the effect of expression of wt ER-α or mutants on bone metastases will be studied in a mouse model. Stable MDA-MB-231 clones from figures 1-3 were inoculated into nude mice to determine the effect of wild-type ER-α or ER-α mutants Ser47Thr, Lys531Glu, and Tyr537Asn on bone and soft tissue metastases.

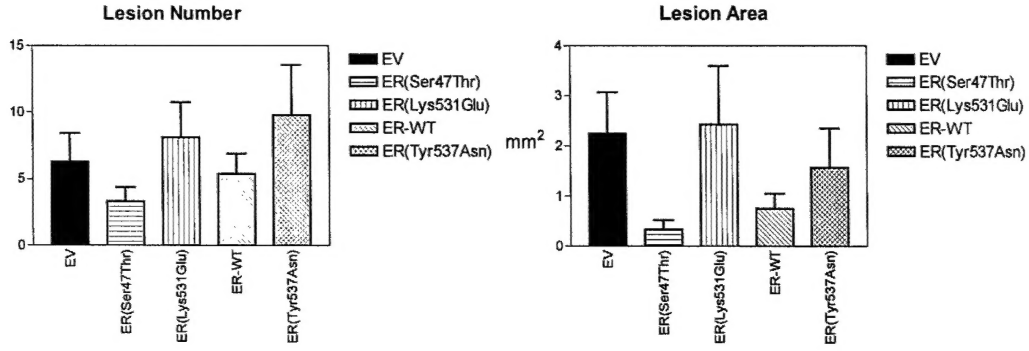


Figure 4: Effect of ER-α, wild-type and mutants, on radiographic bone metastases by MDA-MB-231. Lesion number and lesion area were quantified by computerized image analysis. There was no significant difference in osteolytic lesion number or osteolytic lesion area between MDA-MB-231 clones which express the empty vector, wild-type or ER-α mutants.

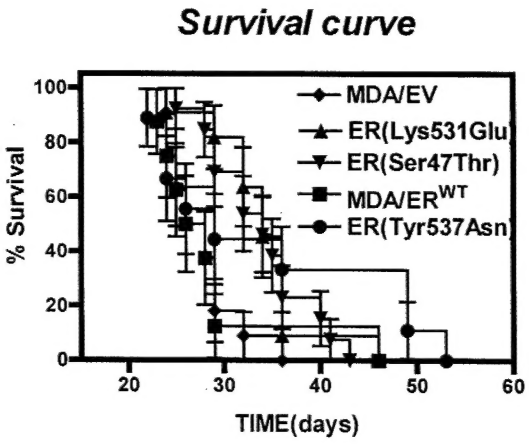


Figure 5: Effect of ER-α, wild-type and mutants, on survival of mice bearing bone metastases by MDA-MB-231. Survival was similar in all groups.

KEY RESEARCH ACCOMPLISHMENTS

- Establishment of stable MDA-MB-231 cell lines which express wild-type ER-α and mutants Ser47Thr, Lys531Glu, and Tyr537Asn.
- Determination that TGFβ increased PTHrP production and ER-α mediated transcription in stable MDA-MB-231 clones which expressed the constitutively active ER-α mutant Tyr537Asn.
- Expression of wild-type ER-α and mutants Ser47Thr, Lys531Glu, and Tyr537Asn by MDA-MB-231 breast cancer had no effect on the development and progression of bone metastases in a mouse model.

REPORTABLE OUTCOMES

Manuscripts, abstracts, presentations:

The following were supported by this Academic Award:

Manuscripts

1. Chirgwin JM, **Guise TA.** Molecular Mechanisms of Tumor :Bone Interactions in Osteolytic Metastases. Critical Reviews in Eukaryotic Gene Expression 12(2):159-178, 2000
2. **Guise TA.** From chondrocytes to cancer: Fibroblast growth factor receptor 3 (FGFR3). Commentary in IBMS BoneKEy 2001 Mar 7 10.1138/ibmske;2001016
3. Bruder JA, **Guise TA,** Mundy GR. Disorders of calcium metabolism. IN: Endocrinology and Metabolism, 4th edition. P. Felig, ed. McGraw Hill Book Company. Chapter 22: 1079-1159, 2001
4. Padalecki SS, **Guise TA.** Actions of bisphosphonates in animal models of breast cancer. Breast Cancer Research. 4(1):35-41, 2002.
5. Mundy GR, Yoneda T, **Guise TA**, Oyajobi B. Local factors in skeletal malignancy. Principles of Bone Biology, 2nd Ed. J Bilezikian, L Raisz, G Rodan, J Markovac. Academic Press Chapter 61:1093-1104, 2002
6. Mundy GR**, Guise TA.** Pathophysiology of bone metastasis, in Cancer and the Skeleton. Edited by Rubens and Mundy. Martin Dunitz Ltd 2000 43-64 2002
7. S-M Käkönen, JM Chirgwin, K Selander, BG Grubbs, JJ Yin, **TA Guise**. Transforming Growth Factorβ (TGFβ) mediates breast cancer osteolysis by stimulating tumor production of parathyroid hormone-related protein (PTHrP) via Smad and MAP kinase pathways. Journal of Biological Chemistry 277(27):24571-8, 2002.
8. Wysolmerski JJ, Dann PR, Zelazny E, Dunbar ME, Insogna KL, **Guise TA**, A Perkins. Overexpression of PTHrP Causes Hypercalcemia, but not Bone Metastases in a Murine Model of Mammary Tumorigenesis Journal of Bone and Mineral Research (7):1164-70, 2002.
9. **Guise TA**, Yin JJ, Thomas RJ, Dallas M, Cui Y, Gillespie MT. Parathyroid hormone-related protein (PTHrP)-(1-139) isoform is efficiently secreted *in vitro* and enhances breast cancer metastasis to bone *in vivo*. Bone 30(5):670-6, 2002.
10. **Guise TA**, Yin JJ, Mohammad K. The role of ET-1 in osteoblastic bone metastases. Cancer In Press (2002)
11. **Guise TA.** Running with Graves' Disease. American Running Association Running & Fitness. 20 (3):7, 2002
12. **Guise TA**. How Metastases Home to Bone: The Attraction of Chemokines. IBMS BoneKEy 2002 Jul 9 0.1138/ibmske;2002052
13. WE Gallwitz, **TA Guise**, GR Mundy. Guanosine nucleotides inhibit the development of osteolytic bone disease due to metastatic breast cancer by their capacity to decrease PTHrP expression. Journal of Clinical Investigation, 110:1559-1772, 2002.
14. **Guise TA**. The vicious cycle of bone metastases. Journal of Musculoskeletal Neuron Interactions 2(6):570-572. 2002
15. Padalecki SS, Weldon KS, Reveles MS**,** Buller C, Grubbs BS, Cui Y, Yin JJ, Hall D, Hummer BT, Weissman BE, Dallas M, **Guise TA,** Leach RJ, Johnson-Pais, T. Chromosome 18 Suppresses Prostate Cancer Metastases. Urologic Oncology. In Press 2003.
16. Lindemann RK, Braig M, Ballschmieter P, **Guise TA,** Nordheim A, Dittmer J. Protein kinase Ca regulates Ets1 transcriptional activity in invasive breast cancer cells. International Journal of Oncology. In Press 2003.

Presentations

1. Molecular mechanisms of osteolytic bone metastases. 2nd North American Conference on "Skeletal Complications of Malignancy." The Paget Foundation-sponsored symposium, Montreal, Canada, October, 1999.
2. Molecular mechanisms of bone metastases. Shriner's Hospital, McGill University, Montreal Canada, October, 1999.
3. Molecular mechanisms of bone metastases: osteolytic and osteoblastic. Endocrine Scholars Lecture Series. University of Connecticut, Farmington, CT, November, 1999.
4. Mechanisms of bone metastases. Department of Cancer Biology Cancer Metastasis Research Program Seminar Series, The University of Texas MD Anderson Cancer Center, Houston, TX, January, 2000.
5. Molecular mechanisms of osteolytic metastases: implications for therapy. Endocrine Grand Rounds. Johns Hopkins University, Baltimore, MD. February, 2000.
6. PTHrP in bone metastases: regulation by TGFβ. Advances in Mineral Metabolism, Snowmass, CO, March, 2000.
7. Role of PTHrP in malignancy. Medicine Grand Rounds, Henry Ford Hospital, Detroit, MI, May, 2000
8. Osteoblastic bone metastases: new insight into mechanisms responsible for bone formation. European Calcified Tissue Society Meeting. Tampere, Finland, May, 2000.
9. Molecular mechanisms of osteolytic bone metastases. Oncology Grand Rounds, University of Michigan, Ann Arbor, MI, July 2000.
10. Cancer and Hypercalcemia. Ashland Endocrine Conference. Ashland, OR, August, 2000.
11. PTHrP as a local mediator of breast cancer osteolysis. Molecular biology of bone working group. American Society of Bone and Mineral Research Meeting, Toronto, Canada, September, 2000.
12. Molecular mechanisms of osteolytic metastases: implications for therapy. Endocrine Grand Rounds, Case Western Reserve University, Cleveland, OH. October, 2000.
13. Breast cancer metastases to bone: role of PTHrP and TGFß. Oncology Grand Rounds, University of Pittsburgh, Pittsburgh, PA. October, 2000.
14. Osteoblastic bone metastases: Role of ET-1. Drug Discovery Lecture Series, University of Pittsburgh, Pittsburgh, PA, October, 2000.
15. Cancer and Bone. Plenary Lecture, First Joint Meeting of the International Bone and Mineral Society and the European Calcified Tissue Society. Madrid, Spain, June 2001.
16. Clinical Insights into biology of the osteoblast. Symposium on novel anabolic approaches to osteoporosis. Endocrine Society Meeting, Denver, CO, June 2001
17. Molecular mechanisms of bone metastases: implications for therapy. Endocrinology Research Seminar, University of Virginia, Charlottesville, VA, July, 2001.
18. Metastatic mechanisms for tumors. Molecular Biology in Orthopaedics. NIH-American Association of Orthopaedic Surgeons Workshop. Scottsdale, AZ, September, 2001.
19. Molecular mechanisms of osteolytic bone metastases due to breast cancer. City of Hope National Medical Center and Beckman Research Institute., Duarte, CA September 2001.
20. Skeletal complications of malignancy. Meet the Professor Session at the American Society for Bone and Mineral Research Meeting, Phoenix, AZ, October, 2001.
21. Skeletal metastases: subversion of normal bone remodeling by cancer cells. Workshop on the Aging Skeleton. American Society for Bone and Mineral Research Meeting, Phoenix, AZ, October, 2001.
22. Molecular Mechanisms of Bone Metastases: Implications for Therapy. Department of

Medicine Grand Rounds, University of Texas Health Science Center at San Antonio, October, 2001.

23. Influence of inflammatory cytokines on bone remodeling in cystic fibrosis. National Meeting for the Cystic Fibrosis Foundation. Orlando, Florida, October, 2001.
24. PTHrP in metastases. Third International Symposium on Cancer-Induced Bone Diseases, Hyogo, Japan, November 2001.
25. Biology and treatment of bone metastasis. New Discoveries in Prostate Cancer Biology and Treatment. American Association for Cancer Research Special Conference. Naples, FL, December, 2001
26. TGFβ in breast cancer metastases to bone. San Antonio Breast Cancer Symposium, San Antonio, TX, December 2001
27. Molecular mechanisms of bone metastases: implications for therapy. Oncology Grand Rounds, Memorial Sloan Kettering, New York, NY, December 2001
28. Molecular mechanisms of bone metastases: implications for therapy. Oncology Grand Rounds, University of California, San Francisco, Breast Cancer Group, San Francisco, CA, March 2002.
29. Molecular mechanisms of bone metastases: implications for therapy. Endocrinology Grand Rounds, Stanford University, Palo Alto, CA March 2002
30. Molecular mechanisms of bone metastases: implications for therapy. Research Seminar, Genentech, South San Francisco, CA, March 2002
31. Role of endothelin-1 in osteoblastic bone metastases. 3rd North American Conference on "Skeletal Complications of Malignancy." The Paget Foundation-NIH sponsored symposium, Bethesda, MD, April 2002
32. Hypercalcemia of Malignancy. Medicine Grand Rounds, East Carolina University, Greenville, NC, April 2002
33. Molecular mechanisms of bone metastases: implications for therapy. Oncology Grand Rounds, Northshore University Hospital, Long Island, NY April 2002
34. Biology of metastasis in bone injury specific to breast cancer. Carroll W. Feist Symposium; Louisiana State University Health Science Center Shreveport, LO. May 2002
35. The vicious cycle of bone metastases. Sun Valley Hard Tissue Workshop, Sun Valley, Idaho, August 2002.

Abstracts

1. JJ Yin, JM Chirgwin, SAW Fuqua, **TA Guise**. Expression of a constitutively active estrogen receptor (ER)- increases PTHrP production and TGFβ-responsiveness by human breast cancer cells. American Society for Bone and Mineral Research Meeting, September, 1999, St. Louis, MO
2. *JJ Yin, BG Grubbs, Y Cui, JR Wu-Wong, J Wessale, **TA Guise**. Osteoblastic bone metastases: tumor-produced endothelin-1 mediates new bone formation via the endothelin a receptor. ET-6, Sixth International Congress on Endothelins, Montreal, Canada, October, 1999.
3. JJ Yin, BG Grubbs, Y Cui, JR Wu-Wong, J Wessale, **TA Guise**. Osteoblastic bone metastases: tumor-produced endothelin-1 mediates new bone formation via the endothelin a receptor. 2nd North American Conference on "Skeletal Complications of Malignancy." The Paget Foundation-sponsored symposium, Montreal, Canada, October, 1999
4. *S-M Käkönen, JM Chirgwin, K Selander, BG Grubbs, JJ Yin, **TA Guise**. Transforming Growth Factor (TGFβ) Stimulates Tumor Production of Parathyroid Hormone-related Protein (PTHrP) via Smad-dependent and –independent mechanisms. European Calcified Tissue Society Meeting, Tampere, Finland, May 2000.
5. *K.S. Selander, S. Reddi, K.W.Harris, E.Valve, P.Härkönen, P.Dean, W.Rankin, **T.A.Guise**, K.Väänänen Increased interleukin-11 expression by breasts cancer cells results in increased bone metastases in mice European Calcified Tissue Society Meeting, Tampere, Finland, May 2000.
6. *JJ Yin, BG Grubbs, Y Cui, JR Wu-Wong, J Wessale, RJ Padley, **TA Guise**. Endothelin A receptor blockade inhibits osteoblastic metastases. American Society for Bone and Mineral Research Meeting, September, 2000, Toronto, Canada.
7. *WE Gallwitz, M Castano, D Horn, G Chapa, S Taylor, **TA Guise**, GR Mundy. Identification of new agents for the treatment of osteolytic bone disease due to metastatic breast cancer by their capacity to decrease PTHrP transcription. American Society for Bone and Mineral Research Meeting, September, 2000, Toronto, Canada.
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CONCLUSIONS

Breast cancer osteolysis is common and the morbidity is devastating. Not only are the consequences of intractable bone pain, fracture, hypercalcemia and nerve compression syndromes debilitating, but the tumor is incurable once it has metastasized to bone. The fact remains that women with breast cancer and bone metastases live many years with this incurable complication and, thus, are at high risk for such morbidity. A more aggressive approach to prevent the development of bone metastases as well as to treat established lesions is a necessary addition to the standard armamentarium for breast cancer therapy in order to impact on this morbidity. Although bisphosphonates are now FDA-approved for treatment of established bone metastases and have had significant impact on bone pain and fracture[6], considerable advances are necessary for the eventual prevention or reversal of bone metastases. These data indicate a central role for TGFβ to potentiate ER-α-mediated transcription induced by a constitutively active ER-α. The above in vitro studies provide rationale for targeting the downstream effects of TGFβ on breast cancer cells to treat and eventually prevent osteolysis. However, in vivo, expression of wild-type ER-α and mutants Ser47Thr, Lys531Glu, and Tyr537Asn

had no effect on the development and progression of bone metastases in a mouse model. Thus, further experiments to test the in vivo relevance of these in vitro findings are warranted.

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